

ANTIOXIDANT ACTIVITY AND NUTRIENT COMPOSITION OF *SORGHUM BICOLOR L.* AND *SECALE CEREALE L.* IN ALGERIA

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ABSTRACT

Whole grain products are recommended for healthy diets as being recognized sources of dietary fibers. In the present study, two types of secondary cereals (rye and sorghum) which are adapted to the growth conditions of Algeria were evaluated for their composition in dietary fibers, sugars, proteins, total phenols and antioxidant properties. Antioxidant activity was evaluated by radical DPPH scavenging capacity, ferric reducing power assay (FRAP) and β carotene-linoleate bleaching assay. The adapted rye grains exhibited better nutritional quality compared to sorghum. Sorghum was exceptionally high in antioxidant activities followed by rye. The nutritional data obtained suggest that the selected grain, particularly sorghum, is promising as healthy food.

KEYWORDS: Rye, Sorghum, Whole Grain, Protein, Dietary Fiber, Total Phenols, Antioxidant Activity

INTRODUCTION

Rye and sorghum can grow and give higher and more stable grain yields in the regions that are characterized by low rainfall or drought, high temperature and low soil fertility. In other words, they perform well under poor soil and growth conditions. The environment and climate in Algeria are characterized by such conditions, and these cereals are adapted in Algeria.

Several reports have shown that sorghum is inexpensive and nutritionally comparable or even superior to major cereals (Duodu et al., 2003). Sorghum grains are also important food cereals in many parts of south Algeria, Asia and the semi-arid tropics worldwide. In Africa, India and China, sorghum grain comes third among cereals for human consumption, super seeded only by rice and wheat (El Khalifa and El Tinay, 2002). In Northern and Eastern Europe, rye is traditional cereal that is generally used as whole meal flour in both soft and crisp breads (Nilsson et al., 1997).

For the development and introduction of these crops to Algeria, there is a need to evaluate their nutritional quality and potential uses as food. Nevertheless, little information is available on the production and quality of these grains in Algeria. Recent studies have shown that cereals grains contain constituents that have demonstrated health benefits for humans, such antioxidant and anti-disease factors (Juntunen et al., 2000; Karppinen et al., 2003; Rieckhoff et al., 1999). For instance, phytic acid was found to play a major role in the treatment of cancer, hypercholesterolemia, hypercalcuria and kidney stones (Plaami, 1997). Other studies have demonstrated that diets high in carbohydrate, rich in dietary fiber, and largely of cereal origin, allowed withdrawal, of oral hypoglycaemic agents or a reduction of insulin dose in diabetic subjects (Pathak et al., 2000). Additionally, several health claims on grain dietary components have been approved by the FDA (Food and Drug Administration) in the USA (Pathak et al., 2000).

Keeping in mind the necessity for increasing dietary fiber and other bioactive dietary components in the diet, additional plant food source are needed. Whole grain products have the potential to make a good contribution in this respect

as recognized sources of dietary fiber, minerals, vitamins and antioxidants, in addition to the proteins and carbohydrates. Therefore, the present study was carried out to determine the nutritional components, and antioxidant activity of these cereals to evaluate their health and nutrition properties, and to encourage the consumption of these cereals in Algeria.

MATERIALS AND METHODS

Grain Materials

Two cereal crops, including sorghum (*Sorghum bicolor* L.), was grown at Adrar in south of Algeria, and rye (*Secale cereale* L) were obtained from ITGC (institut technique des grandes cultures) situated in Sidi Bellabes city in the West Northern of Algeria. Rye was included in the study because it is current by being evaluated for adaptation under the growing condition in Algeria.

The collection of these cereals was given on the level of two region of Algeria in July 2010. At the laboratory, our sample is dried safe from the light then it was preserved in bottles of glasses for later analyses.

Analytical Methods

Chemical Composition

Whole grain cereals were analyzed for moisture, ash and fat according to the Approved Method of the American Association of Cereal Chemists, Method 44–16, Method 08–01, Method 30–10, respectively (AACC, 2003).

The proportioning of total sugars rests on the appreciation of the quantity in monosaccharide present in polysaccharides rests on the proportioning of total sugars by the method of Dubois et al. (1956) also called phenol/acid method.

The proportioning of total nitrogen and the rough proteins are carried out by the classic method of Kjeldahl (1883) by a factor depending on the type of cereal (5.83 for rye and 6.25 for sorghum whole grains).

Total dietary fiber contents were quantified using the method of Henneberg and Stohmann, (1860) using a fiber-extractor (FIWE-VELP SCIENTIFICA).

Polyphenols Dosage

Total phenols content was based on the Folin-Ciocalteu method of Kaluza et al. (1980) using gallic acid as a standard. The reaction mixture contained 250 µl of grain extract, 250 µl of diluted Folin-Ciocalteu reagent and 500 µl of saturated sodium carbonate solution. The mixture was brought up to 5 ml with distilled water, and the contents were mixed and kept in darkness for 30 min. The mixture was centrifuged at 6,000 rpm for 10 min, and the absorbance was read at 725 nm.

The total phenols content was calculated as gallic acid equivalent using the average molar absorptivity of gallic acid. The molar absorptivity of gallic acid was calculated by Beers law using a series of gallic acid concentrations measured under the test conditions.

DPPH• Radical Scavenging Activity

The hydrogen atoms or electron-donating ability of the corresponding extracts was determined from the bleaching of purple-colored methanol solution of DPPH (Hatano et al., 1988).

This spectrophotometric assay uses the stable radical DPPH (2,2-Diphenyl-1-picrylhydrazyl) as a reagent (Burits & Bucar, 2000; Tepe et al., 2005). Radical scavenging activity of extracts was measured by slightly modified method of Mighri et al. (2010) and Braca et al. (2002) as described below.

Different concentrations of each extract were prepared in methanol: 0.2 – 16 mg/ml. A solution of DPPH in methanol (25 µg/ml) was prepared and 2 ml of this solution was added to 50 µl of extract solution in methanol at different concentrations (0.2 – 16 mg/ml). The solution of DPPH was prepared daily before measurements. The sample solutions were shaken vigorously and left standing at room for 60 min in the dark. Then the absorbance was measured at 517 nm using a spectrophotometer (Thermo spectronic He_ios_) against methanol. The blank sample was used as 2 ml of DPPH solution (25 µg/ml in methanol) with 50 µl of methanol. Decreasing of the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity (% of inhibition). This activity is given as percent DPPH radical scavenging, which is calculated with the following equation:

$$\% \text{ DPPH radical scavenging} = [(A_0 - A_t)/A_0] \times 100$$

Where: A₀: Absorbance of blank (t = 0 min);

A_t: Absorbance of tested sample solution at the time t.

The experiment was performed in triplicate and the average absorbance was noted for each measure. The same procedure was followed for the positive control. The methanol was used for baseline correction.

Ferric Reducing Antioxidant Power Assay (FRAP)

The reducing power assay was conducted as previously described by Wang et al. (2008) and Oyaizu (1986) with ascorbic acid (AA) and tert-butyl-4-hydroxyanisole (BHA) being used as the positive controls. In brief, 2.5 ml of individual deionized water diluted grain extracts (ranged from 0.1 to 1 mg/ml) was sequentially mixed with equal volume of phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1% w/v). After incubation at 50°C for 20 min, 2.5 ml of trichloroacetic acid (10 % w/v) was then added to the mixture followed by centrifuging at 3000 rpm for 10 min. Consequently, 5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1 % w/v). After 30 min of incubation at room temperature in the dark, absorbance of the resulting solution was measured at 700 nm using a (Thermo spectronic He_iosisγ).

The ferric reducing power capacities of the grain extracts and standard antioxidants were expressed graphically by plotting absorbance against concentration. Samples for the assay were prepared in triplicate.

β-Carotene-Linoleate Bleaching Assay

The β-carotene bleaching method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (Kulisic et al., 2004). A modified method described by Koleva et al. (2002) was used. β-Carotene (2 mg) was dissolved in 20 ml chloroform and to 4 ml of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40 °C and 100 ml of oxygenated ultra-pure water was added, then the emulsion was vigorously shaken. Reference compound (BHT) and sample extracts were prepared in methanol. The emulsion (3 ml) was added to a tube containing 0.2 ml of extracts. The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50 °C for 120 min, when the absorbance was measured again. BHT was used as positive control. In the negative control, the extract was substituted with an equal volume of methanol. The antioxidant activity (%) of the extracts was evaluated in terms of the bleaching of the β-carotene using the following formula:

$$\text{Inhibition\%} = \frac{A_t - C_t}{C_0 - C_t} \times 100$$

Where: At and Ct are the absorbance values measured for the test sample and control, respectively, after incubation for 120 min, and C0 is the absorbance values for the control measured at zero time during the incubation. The results are expressed as IC50 values ($\mu\text{g/ml}$), the concentration required to 50% β -carotene bleaching inhibition. Tests were carried out in triplicate.

Statistical Analysis

All analyses were carried out in triplicate and the data were reported as means \pm standard deviation. The obtained data were subjected to analysis by One-way ANOVA and the differences between means were at the 5% probability level using Duncan's new multiple range tests using SAS software (SAS, 2001).

RESULTS AND DISCUSSIONS

Nutrient Composition

The major nutrient composition (total sugars, protein, dietary fiber and fat) of cereal whole grain meals are presented in Table 1. Rye had the highest total sugars content averaging approximately 57.75%, compared to sorghum which had total sugars content of about 36.02%. According to Sanaa Ragae (2005), barley and rye contained relatively lower starch contents (53.6% and 58%, respectively) compared to millet and sorghum which had starch contents of about 67.5%. Starch, as the main source of energy in plant foods, is categorized into three types, rapidly digested starch, slowly digested starch and resistant starch on the basis of digestibility. These nutritional starch fractions are different in cereal grains depending upon species, preparation of flours and processing conditions.

Table 1: Chemical Composition (% Dry Basis) of Whole Grain Cereals

	Total Sugars :	Protein :^a	Total Ash :	Crude Fat :	Fiber :
Rye	57.75 \pm 1.8*	13.56 \pm 0.4	2 \pm 0.02	2.16 \pm 0.04*	16 \pm 0.11
Sorghum	36.02 \pm 1.3*	13.03 \pm 0.2	2.22 \pm 0.03	4.67 \pm 0.1*	21 \pm 0.25

^a Nitrogen-to-Protein Conversion Factors are: 5.83 for Rye and 6.25 for Sorghum Whole Grain
Data are the Means \pm Standard Deviations

* Significant Different at ($p \leq 0.05$)

Protein content varied substantially among whole grain meals (**Table 1**). Rye whole grain had the highest protein content, approximately 13.56%, while sorghum whole grain exhibited the lowest level of protein (13.03%) these result are situated in normal values with regard to those published by the FAO in 1995 (proteins: 7 to 15 %). Pomeranz (1981) stated that high nitrogen fertilization, in most instances, increases storage proteins and thus total protein of rye. There are significant difference between each of total sugars and crude fat at ($p \leq 0.05$) while there were no significant difference between the other components.

As expected whole grain sorghum contained higher content of total ash or minerals compared to whole grain rye (Table 1).

Crude fat ranged from 2.16% in rye to 4.67% in sorghum whole grain (Table 1). The high content of fat in whole grain products is due to the presence of embryo in which oil is concentrated. Barley and rye whole grains contained relatively lower fat compared to sorghum and millet (Kaced et al., 1984). The high content of fat in millet (4.2%) should be taken into consideration during storage and processing (Lai & Varriano-Marston, 1980)

In sorghum there is 2.0–4.1% free lipids and 0.1–0.56% bound lipids, the major portion of the lipids is found in the germ (Pomeranz, 1981). Sorghum lipids are highly unsaturated, with oleic and linoleic acids accounting for at least 76% of the total fatty acids (Pomeranz, 1981).

Several publications on nutrient composition in sorghum (Lovis, 2003) and rye (Ragaei et al., 2001), (Gabrovska et al., 2002) are in agreement with the present study.

Total dietary fiber (including resistant starch) was in the following order: sorghum (21%) and rye (16%). Malleshi et al. (1996) reported lower values of total dietary fiber in sorghum (8%), which could be due to different genotypes. Several studies (Ragaei et al., 2001; Gabrovska et al., 2002; Malik et al., 2002; Lovis, 2003) showed that whole grains contain higher concentration of dietary fiber compared to cereals flours and would enhance dietary fiber intake. Joanne et al. (2001) compared chemical composition of wheat flours at different extraction rates (from 66% to 100%). Ragaei et al. (2001) reported higher values of dietary fiber in different rye and triticale flours compared to wheat flour at the same extraction rate. Their results indicate that as the extraction rate increased, all nutrients increased except for starch which decreased with increasing the extraction rate. The results obtained justify using whole grain cereals in bakery products as sources of dietary fiber.

The dietary fiber components in sorghum are mainly cellulose and pentosan. Cellulose level was reported to be 1.19–5.23% (Kamath & Belavady, 1980). The pentosan content of sorghum whole grain ranged from 2.51% to 5.57% depending on variety and environment (Karim & Rooney, 1972). The main dietary fiber fraction in rye is arabinoxylan and it was reported that rye grains contain 9.1% arabinoxylan, 2.3% cellulose, 1.8% β -glucan and 1.2% Klason lignin (Aman et al., 1997).

Total Phenols

Several studies have shown that 80% methanol is an effective solvent in extracting phenolic and other polar substances in cereals (Przybylski et al., 1998; Zielinski & ozłowska, 2000). In this study, 80% methanol extracts from cereals were used for the determination of total phenols content and antioxidant properties. The phenolic compounds are constituted by three big categories: phenolic acids, flavonoids and tannins (Beta, 2003; Dicko et al., 2006; Dykes et al., 2006).

Whole grains significantly differed in total phenols content ranging from 88.49 for rye to 313 $\mu\text{g/g}$ in sorghum whole grains (Table 2). According to Ragaei et al. (2005), sorghum had the highest total phenols content, while barley possessed the lowest content. Millet and rye were intermediate in total phenols compared with sorghum. The total phenols content in barley and oat whole grains was found to be higher than that of wheat and rye and lower than that of buckwheat (Zielinski & Kozłowska, 2000).

Antioxidant Activity

Antioxidant properties of whole grains rye and sorghum were evaluated on the basis of measuring scavenging activity for DPPH radicals, the ferric reducing power assay (FRAP) and β -carotene/linoleic acid systems.

DPPH• Radical Scavenging Activity

In the DPPH test, the colored stable DPPH radical is reduced in the presence of an antioxidant or a hydrogen donor into non-radical DPPH-H, and the reduction in color is monitored over time. The color intensity of DPPH radicals with no antioxidants was stable over the test time.

The free radical scavenging activity is usually expressed as percentage of DPPH inhibition but also by the antioxidant concentration required for 50 % DPPH reduction (IC₅₀). Basically, a higher DPPH radical scavenging activity is associated with a lower IC₅₀ value.

The antioxidant extracts from whole grains rye and sorghum were found to exhibit different reaction kinetics curves compared with the antioxidant standard. The sorghum grain extracts showed a sharp drop in DPPH color intensity and rye extract had relatively the lowest DPPH scavenging capacity compared to sorghum whole grain extracts (**table2**), indicating high antioxidant activity in quenching DPPH radicals and may be due to strong occurrence of polyphenol compounds (flavonoids, tannins, phenols, etc.). Antioxidant activities of cereals extracts are attributed mainly to the compounds present in these cereals.

Significant correlations were observed between total phenols content and DPPH scavenging activity indicating the role of phenolic compounds in inhibiting free radicals under these systems.

The results suggest that phenolic compounds in grains may be able to fight free radicals formed in the human body. Similarly, strong correlations were observed between total phenols and methanolic extracts from whole grain and grain fractions of buckwheat, barley, oat, wheat and rye (Zielinski & Kozłowska, 2000). Statistical analysis showed significant difference in DPPH IC₅₀ and Total phenols between Rye and Sorghum.

Table 2: Phenolic Compounds and Antioxidant Activity Measured by Radicals DPPH of Whole Grain Cereals

Extracts (Polyphenol)	Total Phenols as Gallic Acid Equivalent (µg/g)	DPPH IC ₅₀ (µg/ml)
Rye	88.49 ± 0.70*	11.17 ± 0.50*
Sorghum	313 ± 0.22*	53.83 ± 0.67*

Data are the Means ± Standard Deviations

*Significant Different at (p ≤ 0.05)

β-Carotene/Linoleic Acid Test

In this test, oxidation of linoleic acid produces hydroperoxide-derived free radicals that attack the β-carotene double bonds; the system loses its chromophore and characteristic orange colour resulting in a bleaching of the reaction emulsion. An extract containing antioxidants is able of inhibiting the oxidation of β-carotene by scavenging linoleate-derived free radicals (Jayaprakasha et al., 2001). In the present study, at 0.75 and 1 mg/ml the extract samples for sorghum showed higher ability to prevent bleaching of β-carotene than that of rye samples and those treated by the same concentration (figure 1). The BHA had the most percentage of inhibition with significant difference at (p ≤ 0.05) in compare it with the rye and sorghum, but there are no significant difference appeared among the concentrations.

The inhibition of β-carotene bleaching may be directly linked to the presence of phenolic antioxidants in the tested extracts especially in sorghum grain. The β-carotene bleaching assay only provides an indication of the level of phenols compounds (figure 1). According to Anoma Chandrasekara et al (2012), antioxidant activities of whole millet grains, which was evaluated in the B carotene/linoleate emulsion system, present that extract obtained from this grain inhibited B-carotene oxidation and values ranged from 800 to 1800 antioxidant activity coefficient per g defatted meal. The β-carotene bleaching test (Marco, 1968; Miller, 1971) has been used by many workers to measure the antioxidant activities of plant extracts (Al-Saïxhan et al., 1995; Lee Howard and Villalon, 1995; Auerback and Gray, 1999).

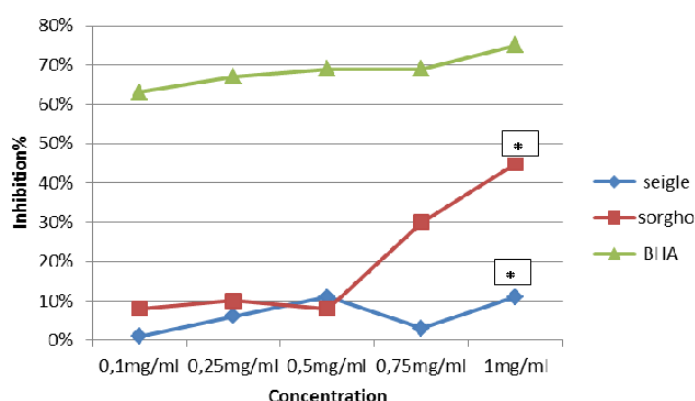
Ferric Reducing Antioxidant Power Assay (FRAP)

Increasing absorbance indicates an increase in reductive ability. Figure 2 presents the dose dependent ferric reducing powers of the sample extracts of sorghum, rye and AA (V.C). The reducing power of the entire sample extracts and AA increased with increasing concentration. The reducing power of AA was significantly more pronounced relative to the cereals extracts at (p ≤ 0.05). However, the antioxidant potencies of AA and sample extracts were comparable at low concentrations. Sorghum extracts have the capacity to act as electron donors, indicating their potential to react with free

radicals, which they can convert to more stable products. For the concentration 0.1 to 1mg/ml, the reducing power of AA and sorghum were higher than that of rye (Figure 2).

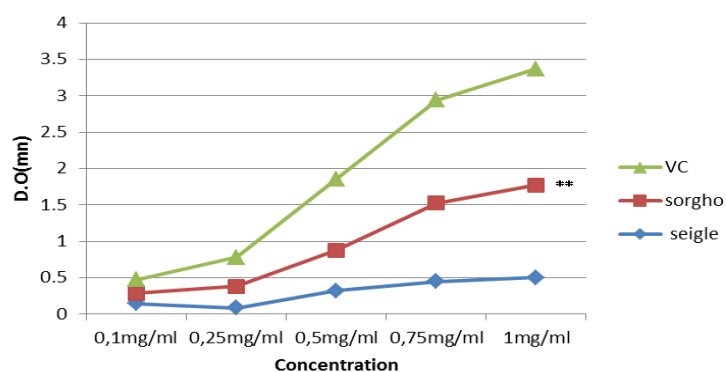
Moreover, for concentrations less than or equal to 0.10 mg/ml, the reducing powers of sorghum was significantly more pronounced than that of AA, generally reducing power was significant different between the concentration each other ($p \leq 0.05$) (Figure 2).

This activity may be due to strong existence of polyphenol compounds such as flavonoids, tannins, and phenols. Due the high total phenolic content of cereals sample extracts; these phenolic compounds represent the primary source of this antioxidant activity.



* Significant at ($p \leq 0.05$) comparing with BHA

Figure 1: B-Carotene/Linoleic Acid Test of Sorghum and Rye Extracts



**Significant at ($p \leq 0.05$) comparing with V.C

*concentration different significant at ($p \leq 0.05$)

Figure 2: Ferric Ion Reducing Power Effects of Sorghum and Rye Extracts, Showing a Dose Dependant Increase in Absorbance

CONCLUSIONS

The role of whole grain products in nutrition and health has been scientifically documented. Whole grains are recognized sources of several physiologically active components and/or health promoters. It has also been well known that bioactive substances found in grains at different concentrations and identities depending on genotypes and phenotypes. Sorghum and rye adapted to Algeria environment were found to contain reasonable levels of nutrients, dietary fiber and antioxidant properties. Incorporation of such materials into bakery products would enhance their nutritional and physiological properties, but farther studies about their organoleptic, technologic properties and acceptability should be taken into consideration.

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